VICAM OCHRATEST
QUANTITATIVE TEST METHOD

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GENERAL INFORMATION

The Vicam Ochratest Quantitative test method is an immunoaffinity column cleanup with fluorometric detection. The test method has been GIPSA approved for quantitative determination of Ochratoxin A in wheat only.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	*Vicam, A Waters Business1-508-482-4935*
Test Kit Name:	Vicam Ochratest Quantitative test kit
Product Number:	13012
Effective Date of Instructions:	5/14/2015
Instructions Revision Number	0
Conformance Range:	5-100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Wheat
Extraction method:	Blend 50 grams sample with 100 milliliters (mL) of 80% Methanol/20% distilled or deionized water on high for 1 minute.
Test Format:	Immunoaffinity column.
Detection Method:	Vicam Series 4 EX only

PREPARATION OF TESTING MATERIALS

a. Phosphate Buffer Saline (PBS).

Prepare the solution by diluting the 100mL 10X PBS concentrate with 900 mL of distilled or deionized water. Prepare this solution monthly or more frequently, if needed.

b. OchraTest Elution Solution.

This solution is supplied prepared, and ready-to-use in a 50 mL amber bottle.

c. <u>Preparation of Extraction Solution A: 80% MeOH/20% deionized or distilled water (v/v) (for Brewers Rice, Brown Rice, Corn, Corn Meal, Milled Rice, Popcorn, Rough Rice, Sorghum, and Wheat):</u>

NOTE: 80/20 (MeOH/water) may be purchased pre-mixed from Vicam (part# 100000211- 20L or part# 100000212-4L). It can also be prepared by following the procedure below.

1. Using a 1000 mL graduated cylinder, measure 800 mL of methanol (ACS grade or better) and place it into a clean carboy with spigot.

2. Using a 250 mL graduated cylinder, measure 200 mL deionized or distilled water and add into the methanol and shake until it is completely mixed.

3. Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.

4. Store this solution at room temperature in a tightly closed container until needed.

d. Fluorometer Calibration

General

An FGIS-approved fluorometer is used to determine the Ochratoxin A level. To ensure accurate results, calibrate the fluorometer prior to use each day and verify using the **Yellow Vial**.

Turn the fluorometer on with the On/Off switch located on the rear panel. Once the fluorometer is turned on, it may be left on until close of business for the day
After turning the fluorometer on, it will identify itself and perform a set of self-tests. If any error message appears, consult the operator's manual.

Follow the procedures listed below to calibrate the fluorometer.

1. Press the "STOP" button. Then press the "OPTIONS" button until "Calibrate Test" is displayed on the screen. Press "ENTER". Press the "SELECT TEST " key until

"OchraTest" is on the screen. Press "ENTER".

2. Follow the prompts on the fluorometer display to calibrate the unit.

3. When prompted to insert a calibration vial, wipe the vial with a clean cloth or paper wipe and insert it into the bottom of the well. Be sure that the vial is fully inserted and touches the bottom of the well.

4. Enter the correct calibration value (see table below) for the high calibrator (red vial) and low calibrator (green vial).

5. Check the calibration by testing the yellow vial. Press "SELECT TEST". Display should read "OchraTest". Then press "ENTER" and insert the yellow vial.

Vicam Series 4EX	
Red	30
Green	-1.3
Yellow	14 +/- 2

6. Record the result for the Yellow Vial.

7. If the value of the yellow calibration vial is not within FGIS specifications, repeat the calibration process (steps a through e listed above), then check the yellow vial again. If the reading for the Yellow Vial remains above or below FGIS specifications, contact Vicam or the Mycotoxin Testing Group at TSD.

8. After the fluorometer is calibrated, place the standards back in the case, close tightly, and store away from any light source.

a. Calibration Standards Maintenance

The standard solutions in the three (3) standard vials (Red, Green, and Yellow) degrade slowly in the presence of light. Store vials in closed case. About 2 months before the expected expiration of the working set, obtain a new set of standards from Vicam.

b. Solution Testing

Prior to beginning test procedures, prepare the solutions required for testing. The PBS solution, distilled/deionized water, and OchraTest elution solution must be tested for background fluorescence before use. After calibrating the fluorometer, perform the following tests in the “Real Time Mode”.

Press "STOP". Press “OPTIONS” button until “Real Time Test” appears on the screen. Press “ENTER”.

1. Place 2.0 mL of PBS into a clean cuvette. Place the cuvette in the calibrated fluorometer.

2. The displayed reading should be less than 0.5 ppb. If the reading is greater than 0.5 ppb, replace the PBS solution.

3. Dispense 1.5 mL of the OchraTest elution solution into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be - 0.5 ppb or less. If the reading is greater than - 0.5 ppb, replace the solution.

4. Place 2.0 mL of distilled/deionized water into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be less than 0 ppb. If the reading is greater than 0 ppb, replace the distilled/deionized water.

5. To continue, press the "STOP" button, then "SELECT TEST". The screen should read "OchraTest". Press "ENTER".

TEST PROCEDURES

a. **Extraction Procedures**

(1) Place 50 grams (+/- 0.2) of ground sample into a blender jar (no salt added).

(2) Add 100 mL of the 80/20 methanol/water extraction solution.

(3) Cover jar and blend at high speed for 1 minute.

(4) Remove the cover and pour the extract into fluted filter paper.

(5) Collect the filtrate in a clean collection container (hard plastic or glass) labeled with sample identification.

b. **Extract dilution and filtration**

(1) Pipette or pour 10.0 mL of the filtered extract into a clean beaker.

(2) Add 40 mL of the PBS solution and mix thoroughly.

(3) Filter the diluted extract through 1.5 µm microfiber filter (Vicam Cat. # 31955) directly into a 10 cc syringe barrel with an OchraTest column attached. Use the markings on the side of the barrel to measure 10 mL.

(4) Immediately proceed with the OchraTest Affinity Column procedure.

c. **Affinity Column**

(1) Remove bottom cap from the Ochratest Column.

(2) Pass 10 mL of the diluted extract completely through the OchraTest column at a rate of about 1 – 2 drops per second until air comes through the column.

Note: Sample analysis using these procedures can be greatly simplified by the use of a small aquarium air pump to provide the needed air pressure for loading, filtering, and washing the various extracts.

(3) Fill the syringe barrel with 10 mL of PBS solution.

(4) Pass the PBS solution through the OchraTest column at a rate of 1 – 2 drops per second.

(5) Fill the syringe barrel with 10 mL of distilled/deionized water.

(6) Pass the water through the column at a rate of 1 – 2 drops per second. Discard waste.

(7) Place a clean glass cuvette under the OchraTest column.

(8) Add/Elute OchraTest column with 1.5 mL of OchraTest eluting solution into the syringe barrel. Pass the solution through the OchraTest column at a rate of 1 drop per second or slower and collect all of the sample eluent (1.5 mL) in the glass cuvette.

(9) Mix well (about 5 seconds) using vortex mixer or by flicking bottom of tube with a finger and **immediately** place the cuvette in a calibrated fluorometer.

(10) After 1 minute (60 seconds), the fluorometer will display the Ochratoxin A concentration. Record the digital readout which will be displayed as total ppb on the work record.

SUPPLEMENTAL ANALYSIS PROCEDURES

There are no supplemental analysis procedures approved for this test method.
Sample results that report above the 100 ppb are certified as “greater than 100 ppb”.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

EQUIPMENT AND SUPPLIES

1. OchraTest affinity columns (Vicam part# 13012).

2. Glass cuvettes (Vicam part# 34000).

3. HPLC grade methanol.

4. Glass microfiber filter paper – (Vicam part# 31955).

5. Distilled or deionized water.

6. Fluted filter paper (Vicam part# 31240).

7. Phosphate buffered saline (PBS) (10X concentrate, Vicam part# G1113).

8. Mycotoxin calibration standards (Vicam part# 33020).

9. OchraTest elution solution (Vicam part# 32016).

10. Blender with stainless steel container.

11. Adjustable pipettor, 200-1000 µL.

12. Pipette tips, 1 mL.
13. Fluorometer – Vicam Series 4EX.

14. Pump assembly stand -Vicam part # G4061 (single position), #21040 (2-position), #21045 (4-position) or #G1104 (12 position).

STORAGE CONDITIONS AND PRECAUTIONS

1. Store OchraTest eluting solution and columns at room temperature (64-86°F, 18-30°C).

2. Do not put the OchraTest eluting solution in a 50 mL bottle dispenser.

3. Keep the OchraTest eluting solution in its original bottle.

4. Do not mix cuvette by putting thumb over top of tube and shaking.

5. Do not collect extracts in soft flexible plastic containers that may leach fluorescent materials.

6. 1.5mL eluting solution can be measured by adding 0.75mL twice.

REVISION HISTORY

Revision 0 (5/14/2015)